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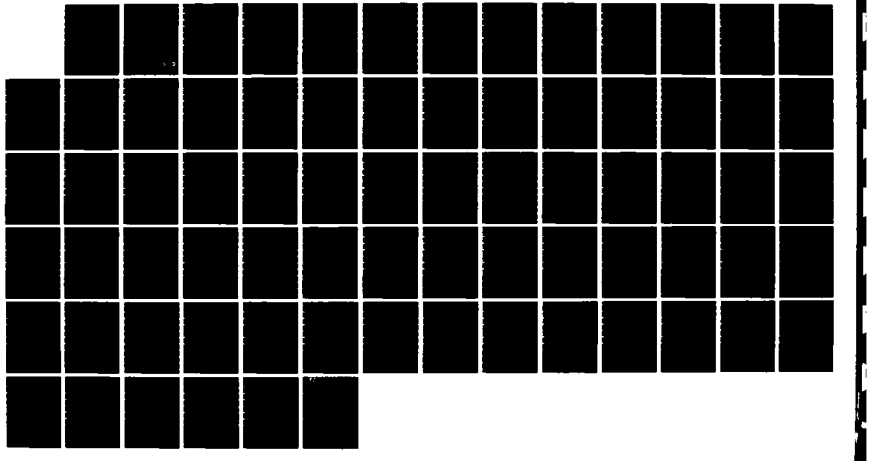

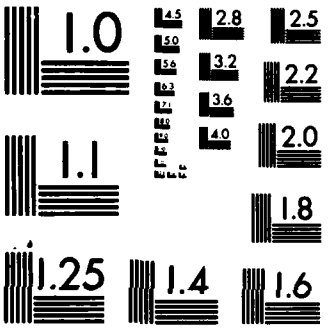

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TECHNICAL REPORT NO. 83-01

EFFECT OF RED BLOOD CELL STORAGE ON CARDIAC PERFORMANCE: IMPROVED MYOCARDIAL OXYGEN DELIVERY AND FUNCTION DURING CONSTANT FLOW CORONARY PERFUSION WITH LOW OXY-HEMOGLOBIN AFFINITY HUMAN RED BLOOD CELLS IN NORMOTHERMIC AND HYPOTHERMIC RABBIT HEARTS

by

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1 February 1983

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Red blood cell storage can result in depression of erythrocyte 2,3 -diphosphoglycerate (DPG) levels, thereby increase oxy-hemoglobin affinity and potentially decrease capillary-to-tissue oxygen transport. We assessed the metabolic and functional effects which resulted from red blood cell storage by using an isolated rabbit heart preparation with an isovolumic left ventricular balloon. Coronary flow was held constant to simulate the physiology of coronary atherosclerosis and other conditions of limited coronary vaso-

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4. CELLS IN NORMOTHERMIC AND HYPOTHERMIC RABBIT HEARTS

20. dilator reserve where coronary flow cannot increase in response to an increase in myocardial oxygen demand. Myocardial function and metabolism were measured under basal conditions and during isoproterenol stress at 37°C and 30°C. The coronary arterial perfusate was alternated between red blood cell suspensions composed of either high or low oxy-hemoglobin affinity red blood cells. The high oxy-hemoglobin affinity state resulted from standard red blood cell storage conditions which resulted in an erythrocyte 2,3-diphosphoglycerate (DPG) level of 0.37 $\pm$ 0.19 umole/gHb and a p50 of 16.9 $\pm$ 0.3 mm Hg at 37 C and a DPG level of 0.94 umole/gHb and p50 of 10.1 $\pm$ 0.2 mm Hg at 30 C. The low oxy-hemoglobin affinity red blood cells were "rejuvenated" by incubation for 60 min in a solution containing pyruvate, inosine, disodium phosphate and adenine which resulted in a restored red blood cell DPG content of 22.7 $\pm$ 2.9 umole/gHb and a p50 of 32.6 $\pm$ 0.5 mm Hg at 37 C and a DPG level of 19.7 $\pm$ 3.9 umole /gHb and p50 of 20.3 $\pm$ 0.3 mm Hg at 30 C. The low oxy-hemoglobin affinity red blood cells resulted in higher levels of myocardial oxygen consumption and contractile function than the high affinity cells during both the basal and stress states at both 37 C and 30 C. Under basal conditions at 37 C, compared to the high oxy-hemoglobin affinity red blood cells, the low oxy-hemoglobin red blood cells increased myocardial oxygen consumption by 29% ($P < 0.005$), coronary venous pO2 by 44% ($P < 0.001$), coronary vascular resistance by 11% ($P < 0.01$), left ventricular developed pressure by 7% ($P < 0.01$), and left ventricular (+) dP/dt by 7% ($P < 0.01$). Isoproterenol stress markedly increased contractile function consumption above basal values for each type fo red blood cell perfusate, but the low oxy-hemoglobin red blood cells resulted in significantly higher levels of function. During isoproterenol stress at 37 C, compared to the high oxy-hemoglobin affinity red blood cells, the low affinity cells increased myocardial oxygen consumption by 29% ($P < 0.005$), coronary venous pO2 by 45% ($P < 0.005$), left ventricular developed pressure by 11% ($P < 0.01$), left ventricular (+) dP/dt by 15% ($P < 0.05$), and the "double-product" of heart rate X developed pressure by 14% ($P < 0.01$). Similar results were observed at 30 C. Under basal conditions, at 30 C, compared to the high affinity red blood cells, the low affinity red blood cells increased myocardial oxygen consumption by 21% ($P < 0.05$), coronary venous pO2 by 67% ($P < 0.001$), coronary vascular resistance by 23% ($P < 0.05$) left ventricular developed pressure by 6% ($P < 0.05$), and left ventricular (+) dP/dt by 11% ($P < 0.05$). During isoproterenol stress at 30 C, compared to the high affinity cells, the low affinity cells increased myocardial oxygen consumption by 25% ($P < 0.005$), coronary venous pO2 by 58% ($P < 0.001$), left ventricular developed pressure by 17% ($P < 0.01$), left ventricular (+) dP/dt by 26% ($P < 0.01$), and the "double-product" of heart rate x developed pressure by 24% ($P < 0.01$). These results demonstrate a significant augmentation of oxygen delivery and contractile function by the "rejuvenated" red blood cells under both normothermic and hypothermic conditions, especially under conditions of hemodynamic stress. Restoration of stored erythrocyte DPG content can potentially benefit patients with coronary atherosclerosis, or other states with a limited coronary vasodilator reserve, who receive large transfusions of stored blood and who are unable to increase coronary arterial flow by a mechanism of vasodilation at a time of increased myocardial oxygen demand.

Effect of Red Blood Cell Storage on Cardiac Performance: Improved Myocardial Oxygen Delivery and Function During Constant Flow Coronary Perfusion With Low Oxy-Hemoglobin Affinity Human Red Blood Cells in Normothermic and Hypothermic Rabbit Hearts*

by

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Short Title: Improved Contractility with Low Oxy-Hb Affinity RBC

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Summary

Red blood cell storage can result in depression of erythrocyte 2,3,- diphosphoglycerate (DPG) levels, thereby increase oxy-hemoglobin affinity and potentially decrease capillary-to-tissue oxygen transport. We assessed the metabolic and functional effects which resulted from red blood cell storage by using an isolated rabbit heart preparation with an isovolumic left ventricular balloon. Coronary flow was held constant to simulate the physiology of coronary atherosclerosis and other conditions of limited coronary vasodilator reserve where coronary flow cannot increase in response to an increase in myocardial oxygen demand. Myocardial function and metabolism were measured under basal conditions and during isoproterenol stress at 37° and 30°C. The coronary arterial perfusate was alternated between red blood cell suspensions composed of either high or low oxy-hemoglobin affinity red blood cells. The high oxy-hemoglobin affinity state resulted from standard red blood cell storage conditions which resulted in an erythrocyte 2,3-diphosphoglycerate (DPG) level of 0.37 ± 0.19 μmole/gHb and a p50 of 16.9±0.3 mm Hg at 37°C and a DPG level of 0.94 μmole/gHb and p50 of 10.1 ± 0.2 mm Hg at 30°C. The low oxy-hemoglobin affinity red blood cells were "rejuvenated" by incubation for 60 min in a solution containing pyruvate, inosine, disodium phosphate and adenine which resulted in a restored red blood cell DPG content of 22.7 ± 2.9 μmole/gHb and a p50 of 32.6 ± 0.5 mm Hg at 37°C and a DPG level of 19.7 ± 3.9 μmole/gHb and p50 of 20.3± 0.3 mm Hg at 30°C. The low oxy-hemoglobin affinity red blood cells resulted in higher levels of myocardial oxygen consumption and contractile function

than the high affinity cells during both the basal and stress states at both 37° and 30°C. Under basal conditions at 37°C, compared to the high oxy-hemoglobin affinity red blood cells, the low oxy-hemoglobin red blood cells increased myocardial oxygen consumption by 29% ($P < 0.005$), coronary venous pO_2 by 44% ($P < 0.001$), coronary vascular resistance by 11% ($P < 0.01$), left ventricular developed pressure by 7% ($P < 0.01$), and left ventricular (+) dP/dt by 7% ($P < 0.01$). Isoproterenol stress markedly increased contractile function consumption above basal values for each type of red blood cell perfusate, but the low oxy-hemoglobin red blood cells resulted in significantly higher levels of function. During isoproterenol stress at 37°C, compared to the high oxy-hemoglobin affinity red blood cells, the low affinity cells increased myocardial oxygen consumption by 29% ($P < 0.005$), coronary venous pO_2 by 45% ($P < 0.005$), left ventricular developed pressure by 11% ($P < 0.01$), left ventricular (+) dP/dt by 15% ($P < 0.05$), and the "double-product" of heart rate X developed pressure by 14% ($P < 0.01$). Similar results were observed at 30°C. Under basal conditions, at 30°C. compared to the high affinity red blood cells, the low affinity red blood cells increased myocardial oxygen consumption by 21% ($P < 0.05$), coronary venous pO_2 by 67% ($P < 0.001$), coronary vascular resistance by 23% ($P < 0.05$) left ventricular developed pressure by 6% ($P < 0.05$), and left ventricular (+) dP/dt by 11% ($P < 0.05$). During isoproterenol stress at 30°C, compared to the high affinity cells, the low affinity cells increased myocardial oxygen consumption by 25% ($P < 0.005$), coronary venous pO_2 by 58% (P 0.001), left ventricular developed pressure by 17% ($P < 0.01$), left ventricular (+) dP/dt by 26% ($P < 0.01$), and the "double-product" of heart rate x developed pressure by 24% ($P < 0.01$). These results demonstrate a significant augmentation

of oxygen delivery and contractile function by the "rejuvenated" red blood cells under both normothermic and hypothermic conditions, especially under conditions of hemodynamic stress. Restoration of stored erythrocyte DPG content can potentially benefit patients with coronary atherosclerosis, or other states with a limited coronary vasodilator reserve, who receive large transfusions of stored blood and who are unable to increase coronary arterial flow by a mechanism of vasodilation at a time of increased myocardial oxygen demand.

Introduction

Large transfusions of stored red blood cells are commonly given to patients during cardiac surgery and for the treatment of hemorrhagic shock. In such patients the oxygen transport function of the transfused erythrocytes may be of particular importance for several reasons. These patients frequently have depressed contractile function, and inadequate myocardial oxygenation can cause further ventricular failure which can become irreversible. Myocardial oxygen demand is often increased in such patients, as a result of the release of endogenous catecholamines and/or their exogenous infusion and because of the tachycardia which is usually present in such cases. Despite the increased myocardial oxygen demand, coronary arterial flow may be decreased in these cases due to arterial hypotension and concomitant decreased coronary perfusion pressure and because of the tachycardia which shortens the diastolic coronary flow period.

Furthermore, coronary flow may be relatively "fixed" in many patients under these circumstances. Normally an increase in myocardial oxygen demand causes coronary arterial vasodilation and an increase in coronary blood flow. However with coronary atherosclerosis and fixed coronary resistances, coronary blood flow may not be able to increase in response to an increase in myocardial oxygen demand, and myocardial ischemia and infarction can occur. Furthermore, in the shock state with arterial hypotension and tachycardia, near maximal coronary vasodilation may occur to compensate for the decreased duration of diastole and reduced

coronary perfusion pressure; consequently, little coronary vasodilator "reserve" may be available in this situation.

Under such conditions, where coronary arterial flow can not adequately increase to keep pace with stress-induced increases in myocardial oxygen demand, the oxygen transport function of the red blood cell and its ability to unload oxygen in passing through the myocardial capillary bed is critically important. Alterations in oxy-hemoglobin affinity have the potential to affect red blood cell oxygen transport function and directly affect myocardial oxygenation. Accordingly, in patients with shock, the physiologic response of oxy-hemoglobin affinity is to decrease in an apparent attempt to maintain oxygen transport and tissue oxygenation despite the reduced arterial flow (Lecompte, et al., 1975; Agostoni, et al., 1975).

However, with transfusions of stored blood, additional factors may be important. The erythrocyte temperature and content of 2,3-diphosphoglycerate (DPG) are major determinants of oxy-hemoglobin affinity state. Under standard blood bank storage conditions the red blood cell DPG content decreases markedly, and oxy-hemoglobin affinity increases. Patients who receive transfusions of stored blood, may undergo a decrease in tissue oxygenation secondary to a shift toward a higher oxy-hemoglobin affinity state in their circulating red blood cell mass, which can persist for 24 hours (Valeri, et al., 1978). Hypothermia also increases oxy-hemoglobin affinity (Benesch, et al., 1969; Hlastala, et al., 1977; Valeri, et al., 1980). Thus the combined effects of hypothermia and

receipt of a large transfusion of stored red blood cells with low 2,3-DPG content would be additive in potentially decreasing myocardial oxygen delivery. This combination of events occurs during open-heart surgery when hypothermia is employed and possibly also when hypothermia and hemorrhagic shock occur concomitantly, and the latter is treated with transfusions of stored red blood cells. Thus patients who are most dependent upon erythocyte oxygen transport function may receive red blood cells which have reduced oxygen transport capacity.

Although the effects of DPG and temperature on oxy-hemoglobin affinity are well-known, the real physiologic consequences of these theorectical changes in oxygen transport remain a subject of some controversy (Bakker, et al., 1978; Woodson, 1979). This controversy has significant practical clinical implications since relatively simple methodology is now available for restoring the stored erythocyte's low DPG content to normal prior to transfusion. (Valeri, et al., 1978). Accordingly, to help resolve this controversy, we developed an experimental model to simulate clinical conditions where coronary flow cannot increase in response to an increase in myocardial oxygen demand. We held the coronary arterial oxygen content and flow rate constant, varied the oxy-hemoglobin affinity state in the coronary arterial perefusate, and measured the effect on myocardial oxygen utilization and contractile function. The effect of oxy-hemoglobin affinity state was assessed under unstressed conditions and also after myocardial oxygen demand was increased with isoproterenol; these comparisons were made at both 37° and 30°C. Our results indicate that red blood cells with restored DPG levels can make a modest but significant improvement in myocardial oxygenation and cardiac function

under these circumstances.

METHODS

In the overall experimental protocol a total of 21 isolated rabbit hearts were perfused for four or eight consecutive 10-15 minute periods, alternating between arterial perfusates containing either high or low oxy-hemoglobin affinity human red blood cells. Contractile function, myocardial oxygen utilization, and myocardial lactate production were measured during each run and the metabolism and performance of the heart were compared during perfusion with each type of red blood cell under basal conditions and during isoproterenol stress. Each heart was exposed to either two or four of the perfusion conditions, and the different affinity and stress states were compared in the same hearts.

The Isolated Perfused Heart.

An isolated normothermic (37°C) or hypothermic (30°C) isovolumic working rabbit heart preparation was used (Figure 1). A small cannulated fluid-filled balloon was placed in the left ventricle and attached to a pressure transducer to monitor intraventricular pressure. Because the balloon was non-compressible, contraction was isovolumic. Since intraventricular volume was held constant, "pre-load" or diastolic fiber length did not change and developed pressure (systolic minus diastolic pressure) and its first derivative (dP/dt) therefore reflected the contractile state of the myocardium.

New Zealand albino male rabbits (1-2 kg) were decapitated, the thorax rapidly opened, and the heart arrested by topical application of chilled saline. The aorta was dissected free, an incision made at the

level of the right innominate artery, and a cannula tied into the root of the aorta. Retrograde coronary perfusion was immediately started with a suspension of washed human red blood cells (see below) so that the time from decapitation to the onset of retrograde coronary perfusion was less than 3 minutes. The left ventricle was decompressed by an apical puncture so that the heart would not be forced to eject against the force of the coronary flow pump. A drain was placed in the apex of the left ventricle so that the chamber remained free of intra-left ventricular fluid from Thebesian drainage. The left atrial appendage was opened and a cannulated collapsed latex balloon (manufactured in our laboratory from the tip of a Trojan-Enz^{R} condom) was inserted into the left ventricular chamber. The balloon cannula was tied in place by a suture around the left atrial appendage. The heart was removed from the thorax and placed in a water-jacketed, constant temperature chamber which was kept at 37^{o} or 30^{o}C with a circulating pump. Intra-cardiac temperature was continuously monitored with a right ventricular probe.

Coronary flow was maintained at a constant rate throughout each experiment with a constant flow roller pump (Technicon Instruments Proportioning Pump Model # 1); due to slight variations in tubing size the coronary flow varied slightly from experiment to experiment. In the experiments done at 37^{o}C (n=12) coronary flow was 6.0 $\pm$ 0.2 and left ventricular wet weight was 3.1 $\pm$ 0.3 gm so that the myocardial perfusion rate was 2.0 $\pm$ 0.1 ml/gm/min. In the experiments done t 30^{o}C (n=9), coronary flow was 5.3 $\pm$ 0.1 ml/min and left ventricular wet weight was 3.0 $\pm$ 0.2 gm so

that mean perfusion rate was 1.80 ± 0.15 ml/gm/min. Thus, in both temperature groups the myocardial perfusion rate was within the physiologic *in vivo* range of 1.8 to 3.4 ml/gm/min for the rabbit (Neutze et al., 1968).

The coronary venous drainage was collected via the cannulated pulmonary artery. All of the rabbit's vena cavae were tied off and a cannula was passed into the body of the right ventricle via the pulmonary artery and tied snugly in place. In this experimental preparation all blood flow into the right side of the heart came from the coronary venous drainage. The coronary venous effluent from the heart was either collected for metabolic analysis or discarded after one passage through the heart and was not re-circulated.

Left ventricular pressure was recorded via a 15 cm length of polyethylene tubing with an internal diameter of 0.045 in. and outside diameter of 0.062 in. (Intramedic Polyethylene Tube PE 160, Clay Adams, Inc., New York, New York). The polyethylene tubing from the ventricular balloon was attached to a Statham P23-Db pressure transducer. A photographic recorder with a high frequency response was used (Electronics for Medicine Model DR8). Left ventricular dP/dt was obtained from the differentiator output circuit of the Electronics for Medicine SGM strain gauge meter/amplifier. The pressure recording characteristics of this system have been previously described (Apstein et al., 1977a; Apstein et al., 1977b).

Aortic root pressure was measured in a similar fashion. In this preparation the aortic root pressure represented the coronary perfusion pressure. Since coronary arterial flow was held constant the coronary perfusion pressure was a direct measure of the coronary vascular resistance.

A pacemaker wire was inserted into the right ventricle via a right atrial incision. The right ventricle was paced at a rate of 180/min with a Grass model S4 stimulator using a 5 msec unipolar impulse with a stimulator voltage adjusted to 0.5V above threshold. In the absence of the isoproterenol infusion (i.e., during the basal state) a pacemaker rate of 180 exceeded the frequency of the endogenous pacemakers and provided a stable constant heart rate. However, during the isoproterenol infusion endogenous pacemaker activity frequently exceeded a heart rate of 180; when this occurred the pacemaker was turned off and the heart was allowed to contract at its spontaneous isoproterenol-stimulated rate.

The collapsed intraventricular balloon was slowly filled with saline while the left ventricular pressure was recorded. The volume of the intraventricular balloon was adjusted to produce a diastolic pressure of approximately 10 mm Hg. Hearts which did not produce a developed pressure of greater than 50 mm Hg at a paced heart rate of 180/min were discarded (approximately 10% of the preparations). Left ventricular pressure, LV dP/dt, and aortic pressure, were monitored continuously throughout each experiment and recorded periodically.

The relationship between balloon size and left ventricular size is critical in this perfusion technique. The balloon must be slightly larger than the ventricle, or else, as the balloon is filled a rise in intra-balloon pressure will be recorded due to an increasing balloon wall tension rather than to ventricular wall tension. A series of balloons of slightly different size was manufactured so that in each experiment the volume of the ventricular cavity was always slightly less than the

balloon capacity. The capacity of each balloon was measured by recording the pressure-volume filling curve of the isolated balloon and the experiments were always performed on the flat portion of the balloon's pressure-volume curve.

The perfusion apparatus is diagrammed in Figure 1. The coronary arterial perfusate reservoir could be switched by simply turning a stop-cock. After passing through the roller pump the suspended red blood cells passed through an oxygenator and a filter of 20 micron pore size (Blood Filter PFF-100, Bentley Laboratories, Inc., Irvine, California 92714) before entering the aortic cannula. The oxygenator was manufactured in our laboratory by coiling approximately 20 ft. of Silastic tubing (0.058 in. I.D. by 0.077 in. O.D., Dow-Corning Corporation Medical Products, Catalog # 602-235, Midland, Michigan) around the outside of a glass beaker. The coiled Silastic tubing was placed inside a larger beaker which was covered with parafilm and gassed with 10-20% O_2, 5% CO_2 and the balance N_2 in order to achieve an arterial pO_2 of approximately 100 mm Hg. The gassing mixture varied slightly from experiment to experiment to produce a consistent arterial pO_2 after passage through the oxygenator. The oxygen content data for the two types of red blood cell perfusates are shown in Table 1.

Preparation of Red Blood Cells with High and Low Oxy-Hemoglobin Affinities.

From healthy human donors, 450 ml of blood was collected into 63 ml of citrate-phosphate-dextrose (CPD) anticoagulant in a triple plastic bag system, concentrated by centrifugation, and stored at 4° C for 22 to 29 days (outdated red cells). Each unit had glycerol added to a final concentration of 40% W/V, the red blood cells were concentrated by centrifugation,

the supernatant was removed, and the red blood cell concentrates were stored in the original collection bag at -80°C for approximately 2 months, until used for these experiments. To make low oxy-hemoglobin affinity red cells, 50 ml of a "rejuvenation" solution containing 100 mmols/L pyruvate, 100 mmols/L inosine, 100 mmols/L disodium phosphate and 5 mmols/L adenine was added and the red cell concentrate was incubated for one hour at 37° C. High oxy-hemoglobin affinity red cells were prepared in an identical manner except they were not incubated with rejuvenation solution. On the morning of a study, units of high and low oxy-hemoglobin affinity red cells were thawed at 42° C for 10 - 12 minutes, washed in 50 ml of 12% sodium chloride and 1.5 liters of 0.9% sodium chloride - 0.2 gm % glucose, and then concentrated by centrifugation to a hematocrit of approximately 90%. For the experiments done at 37°C, the low oxy-hemoglobin affinity (rejuvenated) red cells had a 2,3-DPG level of 22.7 ± 2.9 μmols/gHb and an ATP level of 7.5 ± 0.9 μmols/gHb; the high oxy-hemoglobin affinity red cells had a 2,3-DPG level of 0.37 ± 0.19 μmols/gHb, and an ATP level of 1.57 ± 0.6 μmols/gHb. For the experiments done at 30°C, the low-oxy-hemoglobin affinity (rejuvenated) red cells and a 2,3-DPG level of 19.7 ± 1.3 μmols/gHb and an ATP level of 5.9 ± 0.3 μmols/gHb; the high oxy-hemoglobin affinity red cells had a 2,3-DPG level of 0.94 ± 0.19 μmoles/gHb and an ATP level of 1.2 ± 0.12 μmoles/gHb. The methodology for preparing and freezing the erythrocytes in this manner has been reported (Valeri et al., 1979).

The concentrated red blood cells were resuspended in modified Krebs-Henseleit buffer immediately prior to perfusion into the isolated heart. We wished

to make the two types of red blood cell suspensions equal with regard to the arterial oxygen content; accordingly, we constituted the red blood cell suspensions to have equal hemoglobin concentrations. Because the high affinity oxy-hemoglobin red blood cell volume was slightly larger than the low oxy-hemoglobin affinity cells, the hematocrits of the two red blood cell suspensions were slightly different (see Table 1).

The modified Krebs-Henseleit buffer composition was the same as previously reported (Apstein et al., 1977a; Apstein et al., 1977b) with the following modifications. The buffer contained 2.5 gm albumin/100 ml (Sigma # A-4503, Bovine Albumin, Fraction 5). The albumin was dialyzed against large volumes of Krebs-Henseleit buffer for 24-48 hours prior to use. The glucose concentration in the buffer was 11 mM. The buffer lactate concentration was 1.0 mM, but after mixing with the packed red blood cells the lactate concentration increased, probably due to the glycolytic activity of the red cells (see Table 2). Because albumin binds calcium, it was necessary to readjust the calcium concentration in the perfusate in order to maintain the free, ionized calcium in the physiologic range; calcium chloride was added and the ionized calcium concentration monitored by means of a calcium electrode (Orion Model SS-20) to achieve the ionized calcium concentrations shown in Table 1. Suspension of the red blood cells in the standard Krebs-Henseleit buffer resulted in a hyperkalemic solution, probably because of potassium loss from the red cells. Accordingly, the potassium concentration in the suspending solution was readjusted so that the final potassium concentration, after mixing with the red cells, was in the physiologic range.

The Krebs-Henseleit buffer was passed through a Millipore filter of 1.2 micron pore size (Millipore Corp., Bedford, Mass.) before mixing with the packed red cells. The red cell suspension was passed through an Ultipor^{R} Blood Transfusion Filter (Pall Biomedical Products Corp., Glen Cove, New York 11542) before being utilized for coronary perfusion.

This methodology produced red blood cell suspensions of equal electrolyte composition and arterial oxygen content as shown in Table 1 and allowed the assessment of the oxy-hemoglobin affinity state as the only variable between the two suspensions.

Metabolic Measurements.

Arterial and venous samples were taken at the mid-point of each 10-15 minute perfusion run with each type of red blood cell. An aliquot of blood was deproteinized by mixing 1:1 with 10% trichloracetic acid. The deproteinized supernatant was refrigerated until analysis for lactic acid concentration by a specific enzymatic method (Apstein et al., 1970).

The remainder of the blood sample was drawn into a syringe, capped, kept at 4° C, and immediately analyzed for pO_2, pCO_2 and pH on a blood gas analyzer (Instrumentation Laboratories Model IL-813, Lexington, Mass.), and for total hemoglobin (THb), percent oxy-hemoglobin (Hb O_2%), and hemoglobin oxygen content (Vol % O_2) on an IL 282 CO-Oximeter (8), for total oxygen content (Vol % O_2) on a galvanic fuel cell (Lex O_2 CON-TL Lexington Instruments, Waltham, Mass.), for ionized calcium Ca^{++} on an ion specific electrode (Orion model SS-20, Cambridge, Mass.) and for sodium and potassium concentrations by flame photometry (Instrumentation Laboratories Model 343). The p50, (the oxygen tension at which the hemoglobin is 50% saturated) was obtained by subjecting the red cell

suspension to tonometry at 37° or 30°C at 4 different oxygen tensions in the 40 to 60% saturation range at a pCO_2 of 40 mm Hg with pH corrected to 7.40 (Dennis et al., 1979).

At the end of each experiment the heart was removed from the perfusion apparatus, the right ventricle, atria and large vessels were trimmed, the left ventricle was opened and lightly blotted, and left ventricular wet weight determined.

Experimental Protocol

After the aorta was cannulated and the heart removed from the thorax of the rabbit, coronary perfusion was started with the red blood cell suspension. The sequence of red blood cell type was alternated in consecutive experiments so that the first perfusion run was with the low oxy-hemoglobin affinity cells in half of the experiments and with the high oxy-hemoglobin affinity cells in half of the experiments. Each heart underwent either four or eight sequential perfusion runs. The first perfusion period was of 30 minutes duration, during which time the heart was allowed to stabilize and its initial performance monitored. After the initial 30 minute stabilization perfusion period, the arterial perfusate was switched every 10 to 15 minutes between the high and low oxy-hemoglobin affinity red blood cell suspensions. Four runs were made during the basal state in each heart (i.e. two runs with each type of red blood cell). All 21 hearts were subjected to this experimental protocol comparing the two types of red blood cells during four runs under basal conditions; 12 hearts were kept at 37°C and 9 hearts underwent this protocol at 30°C.

Fifteen of the 21 hearts also underwent an additonal similar comparison

during an isoproterenol infusion, 6 hearts at 37°C and 9 hearts at 30°C. In these 15 hearts, after the four basal state perfusion runs were performed, isoproterenol was infused continuously into the coronary arterial perfusion line to achieve a final isoproterenol concentration of 5 μg/l in the coronary arterial perfusate. After the start of the isoproterenol infusion there was a marked increase in heart rate and contractile function which persisted for 3-4 min. after which the performance stabilized at a "steady-state" level which was less than the peak transient isoproterenol effect, but greater than the pre-isoproterenol level of function. Measurements were made starting at 10 min. after the start of the isoproterenol infusion, when a stable isoproterenol effect was present. During the isoproterenol infusion the heart was switched every 10 to 15 minutes between the two types of red blood cell suspensions. A total of four runs (two with each type of red blood cell) was made during the isoproterenol infusion in each heart. The initial perfusate was alternated so that the isoproterenol perfusion commenced with high oxy-hemoglobin affinity red blood cells in half the experiments and low oxy-hemoglobin affinity red blood cells in half the experiments.

Data Analysis

Mechanical function and metabolic measurements were compared by paired data analysis in each heart. The values obtained during a given run on one type of red blood cell were compared to the values obtained on the runs immediately preceding and following it with the other type of red blood cell. In other words, a given experimental run was compared

to the perfusion runs which bracketed it in time. For each heart we calculated the average change in each parameter which occurred when the perfusate was switched from one type of red blood cell to the other during the basal state and during the isoproterenol stress state. The data were analyzed utilizing the Student t test or Wilcoxon rank test (Snedecor and Cochran, 1967). Data are expressed as the mean $\pm$ the standard error of the mean.

RESULTS

Myocardial Oxygen and Lactate Metabolism

Oxygen Extraction and Utilization at 37°C.

Perfusion with the low oxy-hemoglobin affinity cells at 37°C resulted in a higher rate of myocardial oxygen extraction and utilization relative to the high oxy-hemoglobin affinity cells under basal and stressed conditions (Table 2A). The arterial oxygen content and pO_2 in both perfusates was the same; during perfusion with the low oxy-hemoglobin affinity cells in the basal state myocardial oxygen extraction was 30% higher, myocardial oxygen consumption was 29% higher, and coronary venous pO_2 was 44% higher, presumably reflecting a higher tissue pO_2, during perfusion with the low oxy-hemoglobin affinity cells.

Isoproterenol stimulation increased myocardial oxygen extraction for both types of red blood cell perfusates at 37°C. However, myocardial oxygen consumption per 100gm of LV increased significantly only during perfusion with the low oxy-hemoglobin affinity red blood cells. The low oxy-hemoglobin affinity cells delivered significantly more oxygen to the myocardium during the stressed state than did the high oxy-hemoglobin affinity cells. During the isoproterenol stress and perfusion with the low oxy-hemoglobin affinity cells, relative to the high affinity cells, myocardial oxygen extraction increased by 30%, and myocardial oxygen consumption increased by 29%. During the isoproterenol stress the coronary venous pO_2 remained 45% higher during perfusion with the low oxy-hemoglobin affinity cells than with the high oxy-hemoglobin affinity cells.

These data demonstrate that, at 37°C, under conditions of constant coronary arterial flow and a constant arterial oxygen content, a low oxy-hemoglobin affinity state enhanced oxygen transport to the myocardium

under both basal and stress conditions. Furthermore, when stressed, the myocardium did not significantly increase its oxygen consumption when perfused with the high affinity red blood cells, as it did with the low affinity red blood cells.

Lactate Metabolism at 37°C.

After mixing the packed red blood cells with the Krebs-Henseleit buffer the arterial lactate concentration was approximately 4 mM for both types of red cell perfusates; there was no significant difference in arterial lactate concentration between the high and low oxy-hemoglobin affinity perfusates in either the basal or isoproterenol stress experiments (Table 2A). The group of 12 hearts studied in the basal state at 37°C were in lactate balance and demonstrated neither significant lactate extraction nor production during perfusions with either type of red blood cell suspension. The sub-group of 6 hearts which underwent both the basal state and isoproterenol protcol were also analyzed separately. In these six hearts, in the basal state, significant lactate production occurred during perfusion with the high oxy-hemoglobin affinity red blood cells, but not during perfusion with the low oxy-hemoglobin affinity red blood cells. This result is consistent with the increased oxygen transport provided by the low affinity red blood cells as noted above. During isoproterenol stress modest (8-9%) but significant lactate production occurred during perfusions with both the high and low oxyhemoglobin affinity red blood cells.

Oxygen Extraction and Utilization at 30°C.

Hypothermia did not significantly decrease myocardial oxygen consumption relative to the normothermic state (Table 2B.) Under hypother-

mic conditions, as during normothermia, perfusion with the low oxy-hemoglobin affinity red blood cells resulted in a higher rate of myocardial oxygen extraction and consumption in both the basal and stress states. The arterial oxygen contents and pO_2 values were the same in both types of red blood cell perfusates in the hypothermia experiments. In the basal state, relative to the high oxy-hemoglobin affinity perfusions, the low oxy-hemoglobin affinity red blood cells increased myocardial oxygen extraction by 23% and myocardial oxygen consumption by 21%. The coronary venous pO_2 was 67% higher during the low oxy-hemoglobin affinity perfusions than it was during the high oxy-hemoglobin affinity perfusions, presumably reflecting a higher tissue pO_2 during the low oxy-hemoglobin affinity perfusions.

At 30°C, as occurred at 37°C, isoproterenol stimulation increased myocardial oxygen extraction for both types of red blood cell perfusates; however, myocardial oxygen consumption per 100gm of LV increased significantly only during perfusion with the low oxy-hemoglobin affinity red blood cells. During isoproterenol stress, the low affinity red blood cells delivered significantly more oxygen to the myocardium than did the high oxy-hemoglobin red blood cells. Relative to the high oxy-hemoglobin affinity perfusions, the low oxy-hemoglobin affinity perfusions increased myocardial oxygen extraction by 23%, myocardial oxygen consumption by 25% and the coronary venous pO_2 by 58%, presumably reflecting a higher tissue pO_2.

Thus, these data demonstrate a similar pattern of oxygen metabolism under hypothermic conditions as occurred at 37°C. The low oxy-hemoglobin affinity state enhanced myocardial oxygen transport and consumption at

constant arterial flow and oxygen content in both the basal and stressed states. The stressed myocardium was not able to significantly increase myocardial oxygen consumption when perfused with high affinity red cells, but during perfusion with low affinity red cells, myocardial oxygen consumption increased in response to isoproterenol stress.

Myocardial Lactate Metabolism at 30°C. (Table 2B).

In the hypothermia series there was no significant effect of oxy-hemoglobin affinity on lactate metabolism during the basal state nor during isoproterenol stress. There was neither significant myocardial lactate extraction nor lactate production during both the basal state and isoproterenol stress protocols.

Contractile Function at 37°C.

Contractile function at 37°C was better during perfusion with the low oxy-hemoglobin affinity cells than with the high oxy-hemoglobin cells under both the basal and isoproterenol - stress conditions (Table 3, Figures 2 and 3). Overall cardiac performance was stable during the basal state and first pair of isoproterenol runs. However, during the second pair of isoproterenol runs a significant increase in diastolic pressure occurred, indicating an increase in diastolic LV chamber stiffness or contracture (Apstein et al., 1977b). However, contractile function did not deteriorate.

The absolute data for the entire experimental series at 37°C are presented in Table 3. Paired data analysis was done where each run on one type of red blood cell was compared to the preceding and following run on the other type of cell. The results of the paired runs are presented

in Figures 2 and 3.

During the basal state at 37°C (Figure 2), relative to the high oxy-hemoglobin perfusions runs, the average developed pressure was 7% greater, and left ventricular (+) dp/dt was 7% greater during perfusion with the low oxy-hemoglobin affinity cells. The maximum rate of ventricular relaxation (-) dP/dt, was 8% greater with the low oxy-hemoglobin perfusions than with the high oxy-hemoglobin perfusions. During the basal state the heart rate was held constant at 180/min. Therefore, the double product was proportional to developed pressure (Figure 2).

These data demonstrate a significantly higher level of mechanical performance under basal conditions at 37°C during perfusion with the low oxy-hemoglobin affinity red blood cell suspensions relative to the high affinity oxy-hemoglobin affinity perfusions.

During isoproterenol stress at 37°C, perfusion with the low oxy-hemoglobin affinity red cells also signficantly augmented mechanical function (Figure 3). During perfusion with the low oxy-hemoglobin affinity cells, relative to the high ox-hemoglobin affinity cells, developed pressure was 11% greater and (+) dP/dt was 15% greater. Neither heart rate nor rate of relaxation was significantly affected by oxy-hemoglobin affinity state at 37°C during stress.

Because myocardial oxygen consumption is related to several parameters of myocardial function (see Discussion) we also calculated the "double-product" of heart rate X developed pressure since these two factors are major independent determinants of myocardial oxygen consumption. During the basal state the heart rate was held constant at 180/min. and the double-product was proportional to developed pressure. However, during isoproterenol

infusion, the endogenous heart rate exceeded the external pacemaker's setting of 180/min. During isoproterenol stress the double-product of heart rate X developed pressure was 14% higher during perfusion with the low oxy-hemoglobin affinity cells than it was during perfusion with the high oxy-hemoglobin affinity cells

These data demonstrate a significant augmentation of mechanical function during both basal and stressed conditions at 37°C when the myocardium was perfused with low oxy-hemoglobin affinity cells.

Effect of Isoproterenol on Contractile Function at 37°C.

Isoproterenol markedly increased contractile function at 37°C during perfusions with both types of red blood cells (Fig. 2,3; Table 3). In the six hearts studied in both the basal state and during isoproterenol stress, during perfusions with the high affinity red blood cells, isoproterenol increased LV developed pressure from 65 ± 3 to 74 ± 4 mmHg ($P<0.05$), (+)dP/dt from 1239 ± 85 to 2020 ± 106 mmHg/sec ($P<0.005$), (-)dP/dt from 940 ± 65 to 1694 ± 112 mmHg/sec ($P<0.005$), and the double product of heart rate x developed pressure from 11640 ± 551 to 19345 ± 1759 ($P<0.01$); during perfusions with the low affinity red blood cells, isoproterenol increased LV developed pressure from 68 ± 5 to 82 ± 5 mmHg ($P = 0.05$), (+) dP/dt from 1324 ± 62 to 2317 ± 146 mmHg/sec ($P<0.001$) (-) dP/dt from 1003 ± 38 to 1835 ± 130 mmHg/sec ($P<0.005$) and the double product of heart rate x developed pressure from 12240 ± 822 to 22172 ± 2010 ($P<0.005$). (These values are the average of the two runs in each condition, basal and stress.)

Synergistic Effect of Low Oxy-Hemoglobin Affinity and Isoproterenol on Contractile Function at 37°C.

The isoproterenol infusion produced a greater incremental increase

above the basal level of contractile function when the perfusate consisted of low oxy-hemoglobin affinity red blood cells than when it consisted of high affinity red blood cells. The isoproterenol-induced increase in double product was 7710 ± 1800 during the high oxy-hemoglobin affinity perfusions; during the low oxy-hemoglobin affinity perfusions the addition of isoproterenol increased the double product by 9930 ± 1630 (P 0.05, n=6/group). Thus the presence of the low oxy-hemoglobin affinity cells permitted a greater combined inotropic-chronotropic response to isoproterenol than occurred during perfusion with the high oxy-hemoglobin affinity red blood cells.

The sustained isoproterenol infusion caused an increase in diastolic pressure during runs 7 and 8. Since balloon volume was held constant, the increase in diastolic pressure reflected an increase in diastolic ventricular chamber stiffness (Apstein et al., 1977b). This was not surprising after two hours of perfusion, 30 minutes of which was with isoproterenol. However, contractile function did not deteriorate. In the high affinity perfusions, left ventricular (+) dP/dt in the first isoproterenol run was 2050 ± 129 mmHg/sec and in the second isoproterenol run it was 1982 ± 173 mmHg/sec, P=NS. Likewise, the double product of heart rate x developed pressure was 19990 ± 1680 during the first isoporterenol run and 18680 ± 2630 during the second isoproterenol run (P=NS). Similarly, during the low affinity perfusions, (+)dP/dt was 2429 ± 166 mmHg/sec during the first isoproterenol run and 2204 ± 148 mmHg/sec during the second isoproterenol run (P=NS). These data indicate stability of contractile function throughout the entire protocol. The increase in diastolic stiffness which occurred late in the experiments is reflected by the

increase in ventricular diastolic pressure. During the high affinity perfusions LV diastolic pressure was 9 ± 3 mmHg during the first isoproterenol run and it increased to 17 ± 4 mmHg ($P<0.05$) during the second isoproterenol run. During the low affinity perfusions LV diastolic pressure was 8 ± 2 mmHg during the first isoproterenol run and it increased to 16 ± 3 mmHg ($P<0.05$) during the second isoproterenol run.

Correlation Between Changes in Oxygen Consumption and Mechanical Function on a Heart-by-Heart Basis at 37°C.

The data reported above demonstrate that the low oxy- hemoglobin affinity cells increased the mean level of myocardial oxygen consumption and mechanical function during the basal and stress states at 37°C. However, there was considerable heart-to-heart variation in regard to an individual heart's response to the different oxy-hemoglobin affinity states. To determine whether a given heart's change in mechanical function was proportional to its change in oxygen consumption the data were analyzed on a heart-by-heart basis. The correlation between myocardial oxygen consumption and mechanical function (double-product) is shown in Figure 4. There was a significant correlation between these parameters as the perfusate was alternated between the two oxy-hemoglobin affinity states in both the basal and stress states under normothermic conditions. The change in double-product relative to oxygen consumption was greater during the isoproterenol infusions than during the basal state.

Coronary Vascular Resistance at 37°C.

Since coronary flow was held constant in each experiment, the coronary vascular resistance was measured directly by the coronary perfusion pressure (Figure 1). Coronary vascular resistance was relatively stable throughout the experimental protocol and was consistently higher during the low oxy-hemoglobin affinity runs, indicating a relative degree of coronary vasoconstriction and autoregulation in response to the increase in oxygen availability (Table 3). Under basal conditions, the mean coronary perfusion pressure during the high oxy-hemoglobin affinity runs was 81 ± 10 mm Hg and this increased to 92± 9 mm Hg during the low oxy-hemoglobin affinity runs (+14%, $P < 0.01$). During the isoproterenol infusions the mean coronary perfusion pressure during the high oxy-hemoglobin affinity runs was 82 ± 12 mm Hg; it was 92 ± 8 mm Hg during the low oxy-hemoglobin affinity runs (+12%, P=NS).

Contractile Function at 30°C.

Relative to the normothermic experiments, hypothermia decreased heart rate and increased developed pressure; however the rate of rise of ventricular pressure (+) dP/dt was slightly decreased and the rate of ventricular relaxation (-) dP/dt was markedly decreased (Table 3). The decrease in the rate of rise and fall of ventricular pressure, combined with the increase in developed pressure, had the effect of prolonging the duration of active tension and increasing the area under the left ventricular pressure curve. These effects of hypothermia on myocardial mechanics are well-known (Blinks and Koch-Weser, 1963), and explain why myocardial oxygen consumption was the same at 30°C as it was at 37°C.

During hypothermia, the low oxy-hemoglobin affinity red blood cells increased contractile function in both the basal and stressed states

(Figures 5 and 6, and Table 3). Paired data analysis (Figures 5 and 6) demonstrated that during the basal state with a paced heart rate of 90 beats/min, relative to the high oxy-hemoglobin affinity red blood cells, the low oxy-hemoglobin affinity red blood cells increased developed pressure by 6% and (+) dp/dt by 11%. During isoproterenol stress at 30°C the endogenous heart rate exceeded the pacemaker rate of 90. Relative to the high oxy-hemoglobin affinity red blood cells, the low affinity red blood cells increased developed pressure by 17%, (+) dP/dt by 26%, (-) dP/dt by 12%, and the double-product of heart rate x developed pressure by 24%.

These data demonstrate a significant augmentation of contractile function by the low oxy-hemoglobin affinity red blood cells under hypothermic conditions in both the basal and stress state.

Effect of Isoproterenol on Contractile Function at 30°C.

Isoproterenol markedly increased contractile function at 30°C during perfusions with both types of red blood cells (Figures 5,6; Table 3). During perfusions with the high affinity red blood cells isoproterenol did not increase LV developed pressure (100 $\pm$ 7 vs. 96 $\pm$ 8 mmHg, P=NS). However (+) dP/dt increased from 888 $\pm$ 63 to 1186 $\pm$ 130 mmHg/sec ($P < 0.025$), (-) dP/dt increased from 532 $\pm$ 59 to 722 $\pm$ 108 mmHg/sec ($P < 0.025$), and the double product of heart rate x developed pressure from 9272 $\pm$ 576 to 12478 $\pm$ 1342 ($P < 0.025$). Likewise, during perfusions with the low affinity red blood cells, isoproterenol did not increase LV developed pressure (106 $\pm$ 8 vs. 108 $\pm$ 9 mmHg P=NS). However, isoproterenol increased (+) dP/dt from 982 $\pm$ 80 to 1532 $\pm$ 163 mmHg/sec, ($P < 0.005$), (-) dP/dt from 522 $\pm$ 54 to 823 $\pm$ 107 mmHg/sec ($P < 0.005$), and the double product

from 9855 ± 656 to 15803 + 1473 ($P<0.001$).

Synergistic Effect of Low Oxy-Hemoglobin Affinity and Isoproterenol on Contractile Function at 37°C.

As occurred at 37°C, the isoproterenol infusion produced a greater incremental increase above the basal level of contractile function when the perfusate consisted of low oxy-hemoglobin red blood cells than when it consisted of high affinity red blood cells. The isoproterenol induced increase in double-produce was 3206 ± 982 during the high affinity perfusions; during the low affinity perfusions the addition of isoproterenol increased the double product by 5949 + 1078 ($P<0.001$). Thus the low affinity perfusion permitted a greater chronotropic and intropic response to stress than did the high affinity perfusions.

At 30°C there was less of an increase in LV diastolic pressure during the isoproterenol runs than occurred at 37°C (Table 3). During perfusion with the high affinity cells, during the first isoproterenol run, LV diastolic pressure was 8 ± 1 mmHg and it increased slightly to 11 ± 2 mmHg ($P<0.05$) during the second isoproterenol run. However, during the low affinity perfusions there was no significant increase in LV diastolic pressure between the two isoproterenol runs (8 ± 1 vs. 12 ± 3, P=NS). However, contractile function decreased slightly during the sustained isoproterenol infusion. During the perfusions with the high affinity cells, comparing the first and second isoproterenol runs, LV developed pressure decreased from 94 ± 9 to 85 ± 9 mmHg ($P<0.001$), LV (+) dP/dt did not change significantly, and the double product decreased from 13340 ± 1.20 to 11850 ± 1270 ($P<0.001$). During the low affinity perfusions,

comparing the first and second isoproterenol runs, LV developed pressure decreased from 111 ± 9 to 99 ± 9 mmHg ($P<0.005$), (+) dP/dt decreased from 1586 ± 119 to 1408 ± 146 mmHg/sec ($P<0.005$), and the double product decreased from 16690 ± 1330 to 14510 ± 1350 ($P<0.005$).

Correlation Between Changes in Oxygen Consumption and Mechanical Function on a Heart-by-Heart Basis at 30°C.

Although the mean level of myocardial oxygen consumption and contractile function was greater in the low oxy-hemoglobin affinity group at 30°C. (Figures 5 and 6), significant variation occurred within each group. Accordingly, in figure 7 we plotted the double product as a function of myocardial oxygen consumption for each type of run in each heart under hypothermic conditions. There was a significant correlation between the double-product and oxygen consumption during both the basal and stress states as occurred at 37°C.

Coronary Vascular Resistance at 30°C.

The effect of oxy-hemoglobin affinity state on coronary vascular resistance under hypothermic conditions is shown in figures 5 and 6 in terms of the coronary perfusion pressure which is a direct measure of coronary resistance in this preparation. In the basal state, coronary resistance was 23% higher ($P<0.05$) during the perfusions with low oxy-hemoglobin affinity erythrocytes than during high oxy-hemoglobin affinity perfusions. This increase in coronary resistance indicated a relative degree of coronary vasoconstriction and autoregulation secondary to the increased tissue oxygen delivery provided by the low affinity red blood

cells. During isoproterenol stress we observed a trend (which was not statistically significant) toward an increased coronary resistance during the low oxy-hemoglobin affinity perfusions.

Discussion

In this study we developed an experimental system to test the effects of oxy-hemoglobin affinity state on myocardial contractile function and oxygen metabolism. Under both normothermic and hypothermic conditions, when the hearts were perfused with stored red blood cells whose DPG content had been restored and oxy-hemoglobin affinity decreased, improved contractile function and increased myocardial oxygen consumption was observed compared to perfusion with stored red blood cells with uncorrected low DPG content and high oxy-hemoglobin affinity.

The inverse relationship between erythrocyte 2,3-DPG levels and oxy-hemoglobin affinity is well-known, but its physiologic and therapeutic implications have been questions of controversy (Bakker et al., 1978; Woodson, 1979). The resolution of this controversy is of considerable clinical importance since marked decreases in erythrocyte 2,3-DPG levels occur under standard blood storage conditions, from a normal level of 12-13 to less than 1 umole/gHb after 2-3 weeks of storage. Stored blood has a correspondingly higher oxy-hemoglobin affinity state which has the potential to impair oxygen transport, especially in clinical settings where compensatory mechanisms to maintain oxygen delivery (e.g., arteriolar vasodilation and an increased arterial flow are impaired (Bakker, et al., 1978; Valeri, et al., 1978; Woodson, 1979). Many studies of the effect of affinity state on functional parameters have produced inconclusive or contradictory results, in part due to the difficulty of isolating the oxy-hemoglobin affinity state as the only variable between two experimental groups.

Accordingly we utilized an experimental methodology in which the oxy-hemoglobin affinity state was the only variable. The other major

components of the arterial perfusates were measured and closely matched (Table 1). Coronary flow was held constant in each heart so that auto-regulatory alterations in flow could not compensate for and mask the functional effects of differences in oxygen transport caused by the different affinity states. To minimize experimental variability, the two types of red blood cell perfusates were always compared in the same heart by performing sequential runs and alternating the type of red blood cell; thus each type of red blood cell served as a control for the other type in the same heart.

Our goal was to evaluate the effects of oxy-hemoglobin affinity state per se, not the effects of the metabolic agents which are used to restore the erythrocyte DPG levels. Therefore it was necessary to treat a relatively large volume of red cells in vitro, wash the cells, and re-suspend them before perfusion. This methodology is technically more arduous than altering oxy-hemoglobin affinity state in vivo by infusion of appropriate metabolites, as has been done by others. To accomplish our goal, we utilized human red blood cells which were too old to be given to patients but were available in adequate supply to ensure completion of the study. Methodology for restoring the DPG level in human red cells has been established (Valeri, et al., 1979). Human and rabbit erythrocytes are close to the same size although the human red cell is slightly larger with a mean corpuscular diamter of 7-8μ compared to 6-7μ for the rabbit (Prankerd, 1961). Despite this slight disparity in erythrocyte size between the two species we chose to use the human red cells and the isolated rabbit heart for the reasons outlined above.

This experimental approach was not perfect, but it permitted testing of the oxy-hemoglobin affinity hypothesis. Contractile function was stable for 90 minutes (4 perfusion runs with alternating affinity state) in the basal state and for an additional 30 minutes (two perfusion runs with alternating affinity state) of isoproterenol stress. During another additional 30 min of isoproterenol, deterioration began to occur (Table 3).

The degree of myocardial oxygen extraction ((A-V)/A x 100) in our preparation at the 37°C in the basal state was 24-31% and in the stressed state was 31-41% (Table 2); these values are less than might be expected from studies in man where myocardial oxygen extraction is 60-70% (Messer and Neill, 1962). However, results are consonant with the oxygen extraction and coronary venous oxygen content data reported from other isolated blood-perfused heart preparations. The reasons for the lesser degree of oxygen extraction in isolated hearts are not known, but the lower rate of oxygen extraction relative to the intact human appears to be a relatively consistent observation. For example, the isolated blood-perfused feline heart had an average myocardial oxygen extraction of 46% (range 24-60%) (Vogel et al., 1980). An isolated blood-perfused rat heart preparation was subjected to different coronary perfusion pressures and ventricualr workloads while the arterial pO_2 was kept at 300mmHg; when the isolated rat heart had coronary perfusion pressures and ventricular developed pressures similar to our study, the coronary sinus oxygen saturation averaged 75%, suggesting a myocardial oxygen extraction similar to ours. (Gamble et al., 1970). Likewise, the isolated blood perfused dog heart

in the control state (heart rate = 143, LV systolic pressure = 100mmHg, coronary perfusion pressure = 89mmHg) had an average myocardial oxygen extraction of 29% (Warltier et al., 1976). Thus, our preparation had a pattern of oxygen utilization similar to other isolated blood-perfused heart preparations.

The well-oxygenated myocardium generally extracts lactate from the arterial blood, and we have observed net lactate extraction in isolated rabbit hearts perfused with hemoglobin-free highly oxygenated Krebs-Henseleit buffer (Apstein and Ogilby, 1980; Ogilby and Apstein, 1980; Serizawa et al., 1981). However, the blood-perfused hearts in the current investigation did not demonstrate significant myocardial lactate extraction. In most experimental groups, there was neither significant lactate extraction nor production; however, during isoproterenol stress at 37°C, significant lactate production occurred. Lactate extraction did not occur despite a rate of arterial oxygen delivery (O_2 content x coronary flow) which was several-fold higher than in our buffer-perfused preparation cited above. That overall oxygen delivery was adequate is further attested to by the fact that basal myocardial oxygen extraction was only 24-31% of the arterial content, and myocardial oxygen extraction and contractile function increased with isoproterenol. The level and stability of contractile function in the lactate-producing blood-perfused hearts (Table 3) was comparable to the level of function we have observed in oxygenated buffer-perfused hearts, and, furthermore, the blood-perfused hearts were able to generate a sustained increase in contractile function during the isoproterenol infusion. Hypoxic myocardium can not generate a sustained inotropic response to isoproterenol (Apstein et al., 1976). For these

reasons we believe that the lack of lactate extraction observed in the current investigation does not represent significant ischemia or hypoxia nor deterioration of the preparation. It should also be noted that 11mM glucose was present as substrate, which is twice the concentration used in our previous buffer-perfused studies. This higher arterial glucose level could have decreased myocardial lactate extraction (Williamson, 1962). In any case, the lack of lactate extraction in these studies did not obscure or prevent a beneficial effect of the low oxy-hemoglobin affinity red blood cells on oxygen metabolism and contractile function

This is not an unreasonable preparation in which to test the oxy-hemoglobin affinity hypothesis, since patients who are in shock, or who are undergoing cardiac surgey, and who are likely to receive large transfusions of stored red blood cells, may have borderline or frank myocardial ischemia. In these patients, as in our experimental preparation, prolonged stimulation with adrenergic catecholamines aften leads to mechanical deterioration and increased myocardial lactate production.

Our observations on myocardial lactate metabolism warrant some commentary. Most groups had neither significant lactate extraction nor production (Tables 2A & 2B), however, lactate production occurred in sub-group^{b} when perfused with high affinity cells at 37°C, and during isoproterenol stress at 37°C. Several factors are probably responsible for these patterns of lactate metabolism. The arterial perfusate had 11mM glucose, a substrate known to inhibit lactate utilization by the myocardium and which would have the effect of decreasing net lactate extraction under aerobic conditions. The occurrence of lactate production during the high affinity perfusions at 37°C (sub-group b, Table 2A) and

during isoproterenol stress at 37°C suggests the presence of a mild degree of ischemia under these conditions. The increase in isovolumic LV diastolic pressure during sustained isoproterenol stress is consistent with stress-related ischemia, however, contractile function did not decrease at 37°C, and decreased only slightly during the second isoproterenol run at 30°C. We could have terminated the experiment before the second set of isoproterenol runs, when deterioration occurred, however, we were interested in observing the effect of oxy-hemoglobin affinity state on myocardial function and metabolism when the heart was deteriorating secondary to sustained catecholamine stress, since this parallels the clinical course of many patients who receive large transfusions of stored red blood cells. We were impressed that the relative beneficial effects of the low affinity perfusions were apparent even when we included the data collected as the preparation began to deteriorate.

The relationship between the mechanical function of the heart and its myocardial oxygen demand is a complex one; the three major determinants of myocardial oxygen demand are active tension development, the "contractile state" as assessed by the rate of active tension development, and the frequency of active contractions (Braunwald, 1971). In our isovolumic preparation, where ventricular dimensions are held constant, left ventricular developed pressure is proportional to active tension development. Therefore, in our preparation the "double-product" of developed pressure x heart rate is directly related to myocardial oxygen demand. Our results are consistent with this relationship, especially during isoproterenol stress. Under basal conditions perfusion with the low oxy-hemoglobin affinity cells at both 30°C and 37°C increased myocardial oxygen consumption by 21-29% and the double-product of mechanical function by 6-7%; during

isproterenol stress the low oxy-hemoglobin affinity cells increased myocardial oxygen consumption by 25-29% and the double-product by 14-24%. These changes in myocardial oxygen consumption and double-product were directionally similar but were not precisely proportional probably because the double-product does not account for changes in the "contractile state," which is an additional major determinant of myocardial oxygen consumption. In our preparation maximum (+) dP/dt reflects the contractile state. This parameter increased by 7-11% and 15-26% during the low oxy-hemoglobin affinity perfusions under basal and stress (isoproterenol) conditions respectively. Thus the oxygen cost of the improved contractile state also contributed to the higher rates of myocardial oxygen consumption during the low oxy-hemoglobin affinity perfusions. Thus, when all three major mechanical determinants of oxygen consumption are considered, the changes in mechanics and oxygen consumption where approximately proportional when the two affinity states are compared.

We considered the possibility that the "rejuvenated" (high 2,3-DPG, low oxy-hemoglobin affinity) red blood cells might have some unidentified positive inotropic factor which augmented myocardial function independently of the increase in oxygen availability. This possibility seems unlikely for several reasons. First of all, the red blood cells were washed several times and both types of cells were resuspended in the same batch of Krebs-Henseleit buffer. Secondly, the rejuvenated red blood cells had a synergistic relationship with isproterenol with regard to contractile function. This synergism is consistent with an increase in oxygen availability during the isoproterenol-low oxy-hemoglobin affinity perfusions. If the rejuvenated red blood cells contained an unknown inotropic factor, this inotropic effect should have been manifested equally during both the basal and isoproterenol states. Thus, the observed synergism between

isoproterenol and the low affinity state argues against an "inotropic factor" in the high 2,3-DPG red blood cells and for an increased oxygen transport function as the mechanism for the improved contractile function seen during the low affinity perfusions. However, despite the suggestion noted above, our studies do not definitively prove a causal relationship between the increase in myocardial oxygen consumption and the improved mechanical function observed during perfusion with the low oxy-hemoglobin affinity red blood cells. We believe that the correlation between these two parameters (Figures 4 and 7), and the synergestic effect on contractile function with the isoproterenol and low affinity red cells, provides strong circumstantial evidence that the increased oxygen transport of the low affinity red cells caused the improved contractile performance. However, definitive, direct proof of a cause-and-effect relationship must await further studies.

Our experimental techniques allowed us to demonstrate a beneficial effect of a reduced oxy-hemoglobin affinity state on myocardial function and oxygen metabolism under circumstances where coronary artery flow could not increase. Our results lead to stronger conclusions than previous studies about the potential effect of oxy-hemoglobin state on cardiac function; however several previous studies have provided evidence which tentatively or indirectly supports our conclusions. Rand et al (1979), utilizing an isolated blood-perfused rabbit heart preparation, a rabbit red cell suspension with a hematocrit of 20, and fixed coronary blood flow, demonstrated a significant increase in myocardial oxygen consumption but no significant improvement in mechanical function during perfusion with low oxy-hemoglobin affinity cells during recovery from

hypoxia. During ischemia and post-ischemia recovery, a trend towards increased myocardial function and oxygen consumption was also observed when low oxy-hemoglobin affinity cells were used. Dennis et al (1978) and Weisel et al. (1978) have reported better cardiac function (as assessed by volume loading with measurement of left ventricular Starling curves) in the immediate postoperative period in patients who had received red cells containing higher levels of 2,3-DPG during coronary artery bypass graft surgery (Dennis et al., 1978) or abdominal aortic aneurysectomy (Weisel et al., 1978). In these studies Dennis et al.(1978) compared 2,3-DPG enriched red blood cells to red blood cells with normal levels of 2,3-DPG; Weisel et al. (1978) compared red cells with normal 2,3-DPG levels to red cells with abnormally low values. However, in these two studies unavoidable variations in clinical parameters pre-operatively and during surgery may have contributed to the improved function observed in the lower oxy-hemoglobin affinity groups. Pantely et al. (1981) have recently reported that a lowered oxy-hemoglobin affinity state can reduce myocardial infarct size in the dog; in this study, p50 was altered in vivo by intravenous infusion of dihydroxyacetone, pyruvate and phosphate. Moores et al.(1978) reported a decrease in myocardial function when high oxy-hemoglobin affinity red blood cells were used as prime during right heart by-pass on normoxic swine. Coronary sinus pO_2 increased in this preparation during perfusion with low oxy-hemoglobin affinity cells, but no significant difference in mechanical function occurred. Gross et al. (1977) altered in vivo oxy-hemoglobin affinity state with a 5 min intra-coronary infusion of ortho-iodo sodium benzoate and demonstrated improved myocardial

oxygenation in an isolated canine heart preparation. Jalonen et al. (1980) demonstrated a lower A-V lactate concentration gradient 5 min after coronary reperfusion in patients who had undergone aortic valve replacement when the transfused red cells contained an elevated level of 2,3-DPG. This decrease in lactate production was consistent with improved myocardial oxygenation, but no consistent difference in post-operative myocardial function was demonstrated in the high 2,3-DPG group of patients.

Our experimental preparation and design allowed us to study the effects of oxy-hemoglobin affinity state as altered in vitro; thus we eliminated the in vivo infusion of metabolites as an additional experimental factor. The isolated heart preparation allowed us to elimiate the variables which have complicated many previous studies and to measure precisely the important parameters of cardiac function which are related to myocardial oxygen consumption. Under these circumstances significant beneficial effects of the low oxy-hemoglobin affinity red blood cells were apparent.

Our results suggest certain clinical circumstances under which the use of red blood cell transfusions with a low oxy-hemoglobin affinity state may be particularly important. Patients who receive large transfusions after trauma, hemorrhage, or during cardiac surgery are usually in a stressed condition, have an increased level of circulating catecholamines due to endogenous production and/or exogenous infusion, and may be hypothermic. These patients may be anemic, may have some degree of coronary atherosclerosis, since this disease is so common, and may have limited coronary vasodialtor reserve. This combination of circumstances is quite similar to our experiments where coronary arterial flow was held constant and myocardial oxygen demand stimulated with isoproterenol.

Under these conditions the beneficial effects of low oxy-hemoglobin affinity red blood cells were most apparent in their augmentation of both myocardial oxygen delivery and contractile function. We conclude that use of erythrocytes with a restored 2,3-DPG level might be of significant benefit under these clinical circumstances.

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Figure Legends

Figure 1. Isolated Heart Perfusion System

The isolated rabbit heart was suspended in a water-jacketed constant temperature chamber. A thin-walled fluid-filled latex balloon occupied the left ventricular cavity. A cannula from the balloon passed across the mitral valve orifice, through the left atrial appendage, to monitor left ventricular pressure. A temperature probe and pacing wire were passed into the right ventricular cavity via the right atrium. The coronary arterial perfusate consisted of either high or low oxy-hemoglobin affinity red blood cell suspensions as described in the text. A stop-cock position determined which type of red blood cell perfusate entered the perfusion system; the pump, perfusion lines, and time spent in passage through the oxygenator were identical for both types of red blood cell perfusates. After passage through the oxygenator the red cell perfusate passed through a Bentley PFF-100 filter. Since coronary flow rate was held constant the mean aortic pressure (coronary perfusion pressure) was directly proportional to the coronary vascular resistance. A syringe infusion pump was utilized in some experiments to deliver isoproterenol into the perfusion line. In this preparation the coronary circulation comprises all of the flow into the right side of the heart; the pulmonary artery was cannulated to collect the coronary venous drainage. A small drain was placed across the apex of the left ventricle to permit escape of Thebesian drainage from the left ventricular cavity.

Figure 2. Effect of Oxy-Hemoglobin Affinity State on Myocardial Function Under Basal Conditions at 37°C.

Each point represents the average of two runs during the basal state. The lines connect values obtained in the same heart.

Figure 3. Effect of Oxy-hemoglobin Affinity State on Myocardial Function During Isoproterenol Stress at 37°C.

Each point represents the average of two runs during isoproterenol stress. The lines connect values obtained in the same heart.

Figure 4. Relationship Between Cardiac Function and Myocardial Oxygen Consumption at 37°C.

Cardiac function, as assessed by the double-product of heart rate x left ventricular developed pressure, is plotted as a function of myocardial oxygen consumption.

Each point represents the average of two runs in each heart. Runs done in the basal state are indicated by (●) for high oxy-hemoglobin affinity perfusions and by (0) for low oxy-hemoglobin affinity perfusions. Runs done during isoproterenol stress are indicated by (▲) for high oxy-hemoglobin affinity perfusions and by (△) for low oxy-hemoglobin affinity perfusions. The points with the standard error bars represent the means ±SEM for each experimental group. Each line represents a least-square linear regression for the points obtained during the basal and isoproterenol stress states. During the basal state $Y=437X + 8554$, $r=0.6124$, $P < 0.005$. During the isoproterenol stress state $Y=1212X + 9788$, $r=0.8623$, $P < 0.001$. The slopes of the two lines were significantly different at $P < 0.01$ (t test). These plots indicate that the changes in

oxygen consumption which occurred during perfusion with red blood cells with different oxy-hemoglobin affinity states were associated with a proportional change in cardiac function during both the basal and isoproterenol stress conditions at 37°C.

Figure 5. Effect of Oxy-Hemoglobin Affinity State on Myocardial Function Under Basal Conditions at 30°C.

Each point represents the average of two runs during the basal state; the lines connect the values obtained in the same heart. (*:$P < 0.05$).

Figure 6. Effect of Oxy-Hemoglobin Affinity State on Myocardial Function During Isoproterenol Stress at 30°C.

Each point represents the average of two runs during isoproterenol stress; the lines connect values obtained in the same heart. (**: $P < 0.01$; *: $P < 0.05$).

Figure 7. Relationship Between Cardiac Function and Myocardial Oxygen Consumption at 30°C.

Cardiac function, as assessed by the double-product of heart rate x left ventricular developed pressure (Rate x Pressure Product) is plotted as a function of myocardial oxygen consumption. Each point represents the average of two runs in each heart. The points with the standard error bars represent the means $\pm$ SEM for each group. Each line represents a least-square linear regression for the points obtained during the basal and isoproterenol stress states. During the basal state, $Y = 707X + 4872$, $r=0.6347$, $P<0.01$. During isoproterenol stress $Y = 1009X +$

5865, r = 0.6989, $P < 0.005$. There was no significant difference between the slopes of the two lines. These plots indicate that the changes in oxygen consumption which occurred during perfusion with red blood cells with different oxy-hemoglobin affinity states were associated with a proportional change in cardiac function during both the basal and isoproterenol stress conditions at 30°C.

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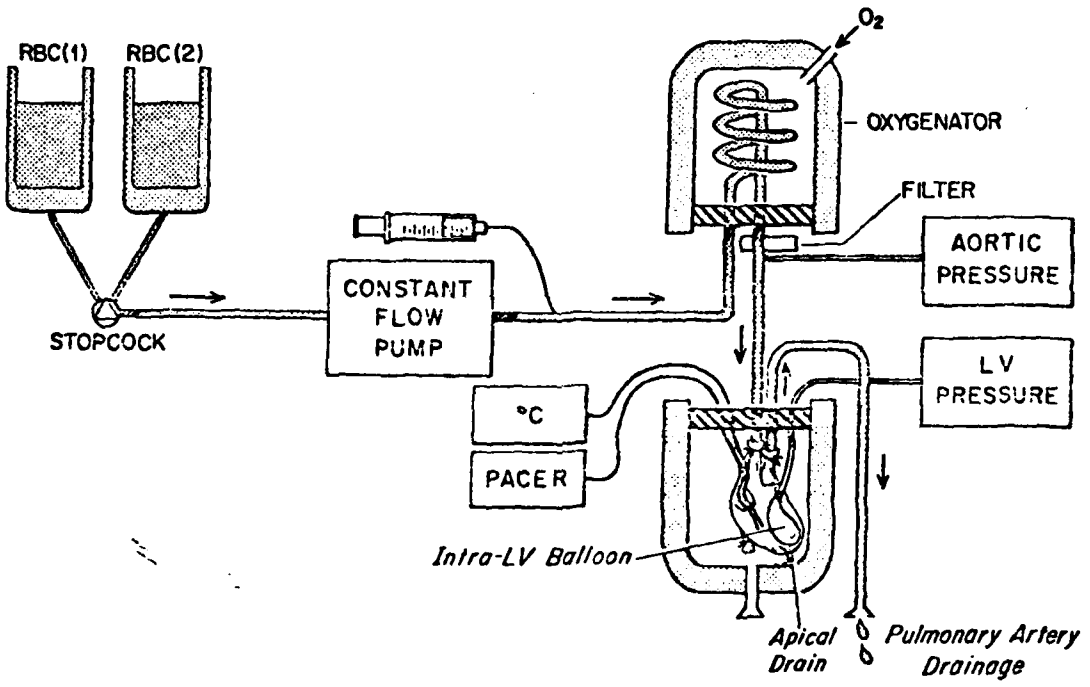

RBC(1)
RBC(2)
STOPCOCK
CONSTANT FLOW PUMP
O_2
OXYGENATOR
FILTER
AORTIC PRESSURE
LV PRESSURE
°C
PACER
Intra-LV Balloon
Apical Drain
Pulmonary Artery Drainage

FIG. 1

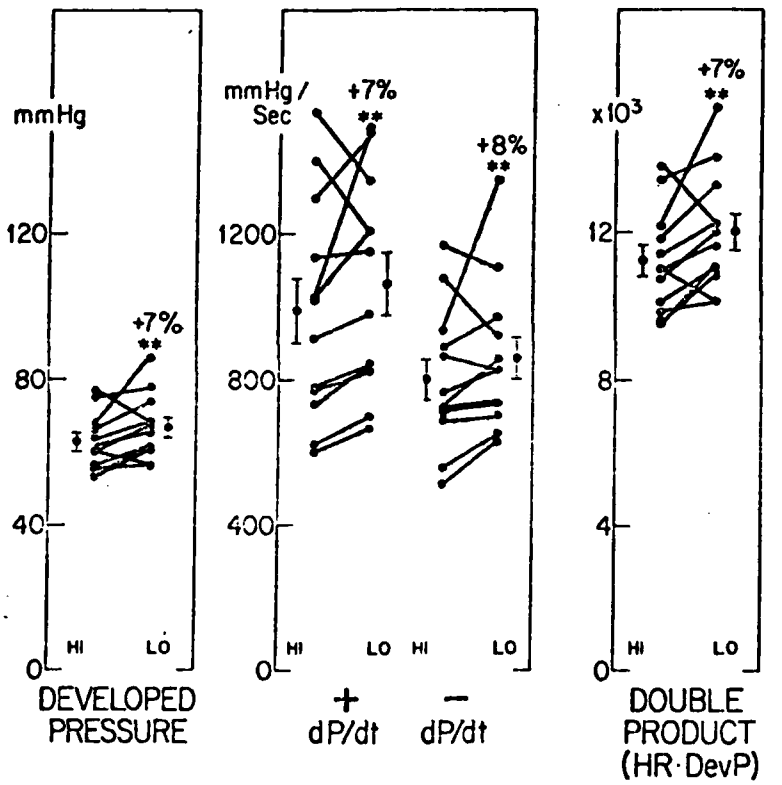

mmHg
120
80
40
0
+7%
**
HI
LO
DEVELOPED PRESSURE
mmHg / Sec
1200
800
400
0
+7%
**
+8%
**
HI
LO
HI
LO
+ dP/dt
− dP/dt
x10³
12
8
4
0
+7%
**
HI
LO
DOUBLE PRODUCT (HR·DevP)
HI: High Aff. Oxy-Hb
LO: Low Aff. Oxy-Hb
**P<0.01

FIG. 2

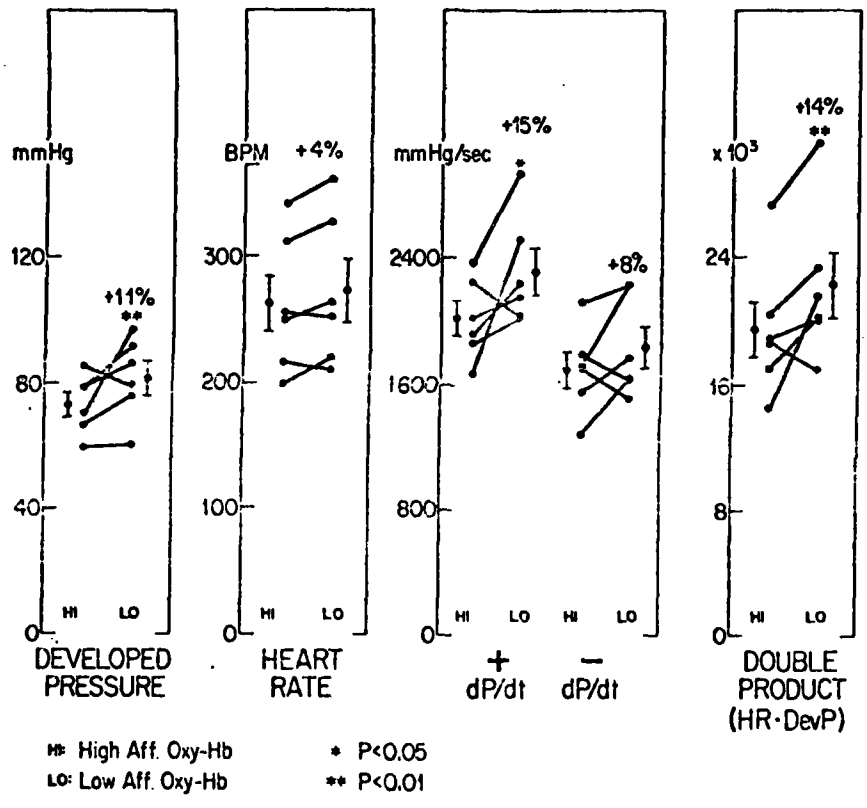

mmHg
120
80
40
0
+11%
**
HI
LO
DEVELOPED PRESSURE
BPM
+4%
300
200
100
0
HI
LO
HEART RATE
mmHg/sec
+15%
*
2400
1600
800
0
HI
LO
+
dP/dt
+8%
HI
LO
−
dP/dt
x 10³
+14%
**
24
16
8
0
HI
LO
DOUBLE PRODUCT (HR·DevP)
HI: High Aff. Oxy-Hb
LO: Low Aff. Oxy-Hb
* P<0.05
** P<0.01

FIG. 3

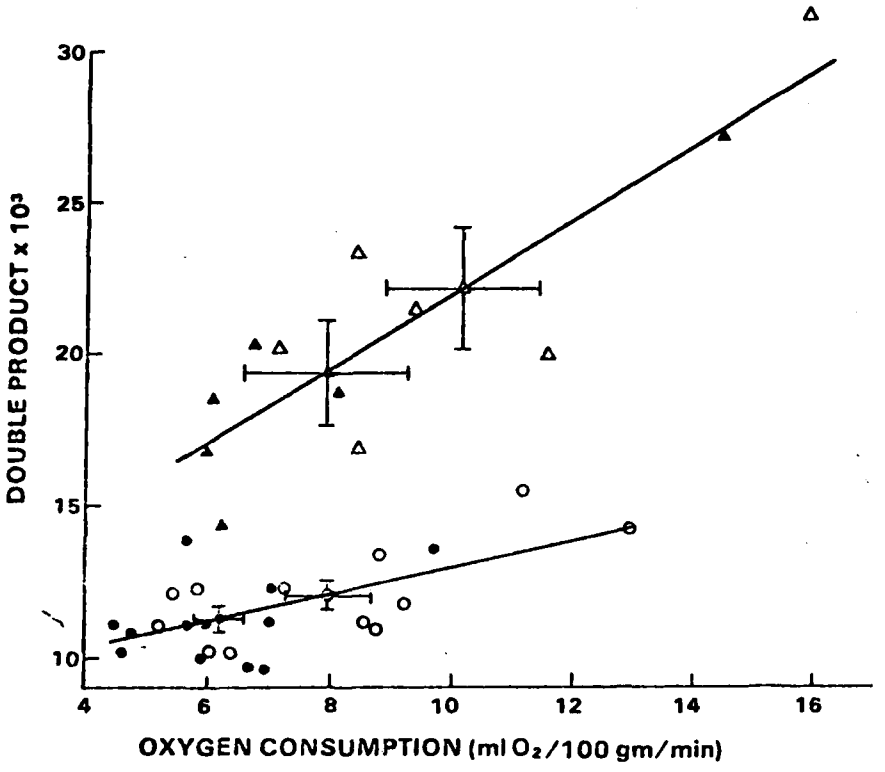
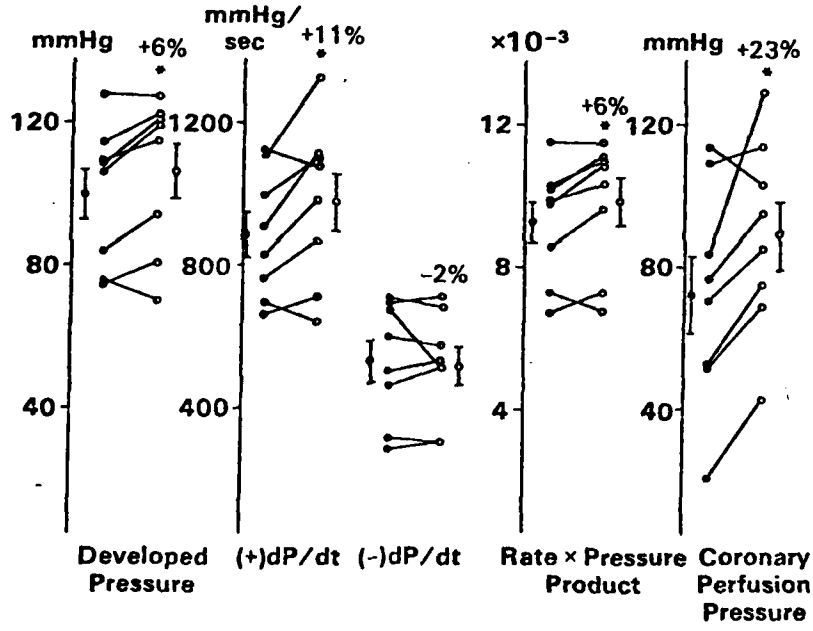

DOUBLE PRODUCT x 10³
30
25
20
15
10
4
6
8
10
12
14
16
OXYGEN CONSUMPTION (ml O₂/100 gm/min)

FIG. 4

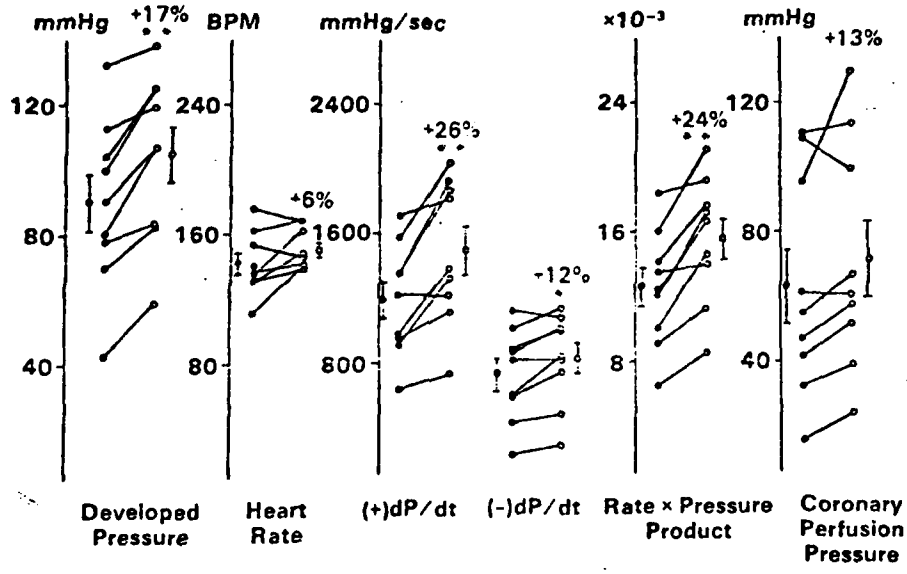
Effect of Oxy-Hb Affinity on Cardiac Function at 30° C. During Basal State



(●- High Aff. Oxy-Hb; ○-Low Aff. Oxy-Hb)

Fig. 5

Effect of Oxy-Hb Affinity on Cardiac Function at 30° C. During Isoproterenol Stress



(●- High Aff. Oxy-Hb; o- Low Aff. Oxy-Hb)

Fig. 6

Effect of Oxy-Hb Affinity on Cardiac Function at 30 ° C.

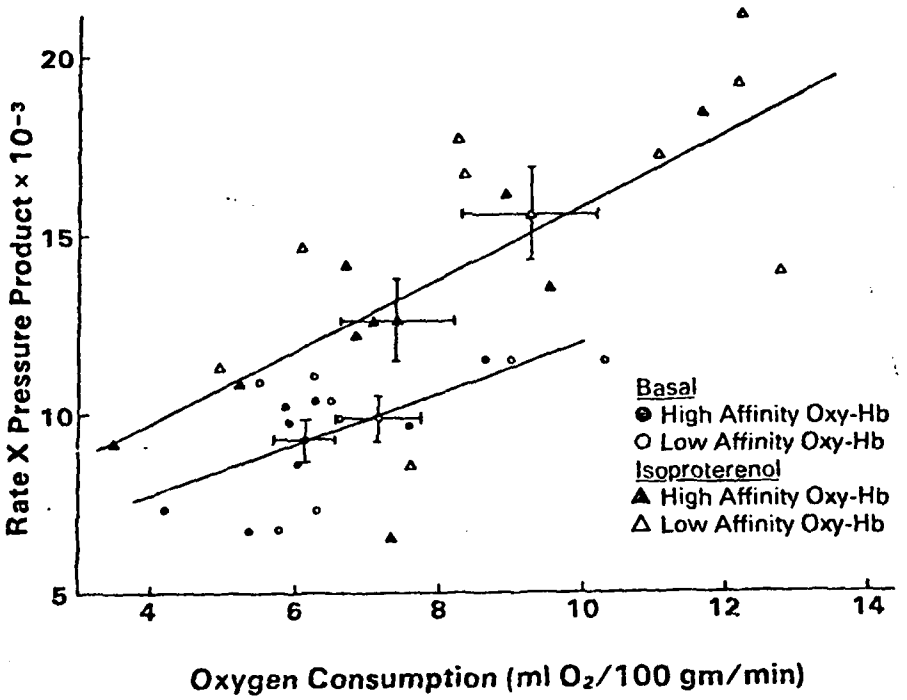


Fig. 7

TABLE 1

ARTERIAL BLOOD COMPOSITION

Normothermia Series (37°C)

	High-Affinity Oxy-Hb (n=12)		Low-Affinity Oxy-Hb (n=12		P
p^{50} (mm Hg)	16.9	±0.3	32.6	±0.5	<0.001
Hct	31.2	±0.4	29.5	±0.4	<0.001
Hb (gm%)	10.3	±0.2	10.3	±0.2	NS
$p^{0}2$	100.9	±11.8	93.0	±6.1	NS
% Oxy-Hb Sat.	96.1	±0.7	93.6	±1.0	NS
0_2 Content (Vol.%)	13.7	±0.3	13.4	±0.4	NS
Ca++ (ionized, mEq/l)	2.24	±0.05	2.29	±0.09	NS
K+ (mEq/l)	5.0	±0.3	4.9	±0.3	NS
Na+ (mEq/l)	136	±1.3	135	±1.6	NS
pH	7.381	±0.012	7.376	±0.011	NS

Hypothermia Series (30°C)	High Affinity Oxy-Hb (n=9)		Low Affinity Oxy-Hb (n=9)		P
p50 (mm Hg)	10.1	±0.2	20.3	±0.3	<0.001
Hct	31.1	±0.3	29.9	±0.3	<0.025
Hb (gm%)	10.2	±0.1	10.2	±0.1	NS
p02	62.2	±7.0	72.6	±6.4	NS
% Oxy-Hb Sat.	95.4	±0.8	95.3	±0.6	NS
0_2 Content (Vol.%)	13.6	±0.2	13.5	±0.2	NS
Ca++ (ionized, mEq/l	2.80	±0.21	2.73	±0.23	NS
K^+ (mEq/l)	5.0	±0.3	4.9	±0.4	NS
Na^+ (mEq/l)	135.0	±1.4	133.8	±0.8	NS
pH	7.466	±0.021	7.453	±0.028	NS

TABLE 2A

MYOCARDIAL OXYGEN AND LACTATE METABOLISM

Normothermia Series (37°C)

Basal State[a] (n=12)	High Oxy-Hb Affinity	Low Oxy-Hb Affinity	%Change	p[t]
Arterial pO2 (mm Hg)	100.9 ± 11.8	93.0 ± 6.1		NS
Venous pO_2 (mm Hg)	28.8 ± 1.8	41.6 ± 2.7	+44%	<0.001
Arterial % Oxy-Hb Sat.	96.1 ± 0.7	93.6 ± 1.0		NS
Venous % Oxy-Hb Sat.	72.5 ± 1.8	64.4 ± 1.9	-11%	<0.001
Arterial O_2 Content (Vol %)	13.7 ± 0.3	13.4 ± 0.4		NS
Venous O_2 Content (Vol %)	10.5 ± 0.4	9.2 ± 0.3	-12%	<0.001
% O_2 Extraction $(\frac{A\ V}{A} \times 100)$	24.0 ± 1.8	31.3 ± 2.3	+30%	<0.005
Myocardial O_2 Consumption (ml/min/100 gm)	6.2 ± 0.4	8.0 ± 0.7	+29%	<0.005
Arterial Lactate Conc. (mM)	4.00 ± 0.18 } #	3.79 ± 0.23 } #		NS
Venous Lactate Conc. (mM)	4.15 ± 0.16 } #	3.90 ± 0.21 } #		NS
A-V (mM)	-0.04 ± 0.08	-0.05 ± 0.09		NS
% Lactate Extraction $(\frac{A-V}{A} \times 100)$	-4.3 ± 1.9	-3.7 ± 2.9		NS

Basal State[b] (n=6)				
Arterial pO_2 (mm Hg)	94.7 ± 17.1	91.2 ± 6.1		NS
Venous pO_2 (mm Hg)	29.5 ± 3.2	38.6 ± 4.8	+31%	<0.05
Arterial % Oxy-Hb Sat.	95.9 ± 1.2	94.6 ± 1.3		NS
Venous % Oxy-Hb Sat.	74.6 ± 2.3	66.1 ± 2.1	-11%	<0.025
Arterial O_2 Content (Vol %)	14.4 ± 0.2	14.0 ± 0.2		NS
Venous O_2 Content (Vol %)	11.3 ± 0.3	9.7 ± 0.3		NS
% O_2 Extraction (A-V)/A x 100	21.7 ± 2.1	30.4 ± 3.1	+40%	<0.025
Myocardial O_2 consumption (ml/min/100 gm)	5.6 ± 0.4	7.5 ± 0.9	+34%	<0.05
Arterial Lactate Conc. (mM)	3.80 ± 0.31 }	3.25 ± 0.29 }		NS
Venous Lactate Conc. (mM)	4.03 ± 0.29 } *	3.52 ± 0.30 } NS		NS
A-V (mM)	-0.23 ± 0.06	-0.27 ± 0.12		NS
% Lactate Extraction (A-V/A X 100)	-6.1 ± 1.6	-8.3 ± 3.7		NS

TABLE 2A (Cont.)

Isoproterenol Stress[b]	High Oxy-Hb Affinity	Low Oxy-Hb Affinity	%Change	p[t]
Arterial pO2 (mm Hg)	97.7 ± 11.4#	100.3 ± 6.5*		NS
Venous pO2 (mm Hg)	26.6 ± 1.0#	38.7 ± 1.7#	+45%	<0.005
Arterial % Oxy-Hb Sat.	96.8 ± 0.6#	95.9 ± 0.8#		NS
Venous % Oxy-Hb Sat.	66.1 ± 2.6**	56.2 ± 2.5**	-15%	<0.025
Arterial O_2 Content (Vol %)	13.6 ± 0.1***	13.4 ± 0.1**		NS
Venous O_2 Content (Vol %)	9.3 ± 0.3***	7.9 ± 0.3***	-15%	<0.001
% O_2 Extraction ($\frac{A\ V}{A}$ X 100)	31.4 ± 2.6**	40.9 ± 2.7***	+30%	<0.001
Myocardial O_2 Consumption (ml/min/100 gm)	7.9 ± 1.4#	10.2 ± 1.3*	+29%	<0.005
Arterial Lactate Conc. (mM)	4.21± 0.28 } **	3.80± 0.23 } *		NS
Venous Lactate Conc. (mM)	4.59± 0.30 } *	4.13± 0.30 } *	-10%	<0.05
A-V (mM)	-0.38± 0.04	-0.33± 0.10		NS
% Lactate Extraction($\frac{A\ V}{A}$ X 100)	-9.17± 0.9	-8.3 ± 2.4		NS

t: P for Low vs. High Oxy-Hb Affinity, paired *t* test

a: N=All 12 hearts in basal state, 37°C experiments

b: N=6 hearts subjected to basal state protocol followed by isoproterenol protocol; for this series for isoproterenol vs. no isoproterenol, # is P=NS, * is P 0.025, ** is P 0.01, *** is P 0.001, by paired t test. Also, # is P=NS, * is P 0.025, *** is P 0.001 for art. vs. venous lactate conc.

TABLE 2B

MYOCARDIAL OXYGEN AND LACTATE METABOLISM AT 30°C.

Hypothermia Series (30°C)

Basal State (n=9)	High Oxy-Hb Affinity	Low Oxy-Hb Affinity	% Change	pt
Arterial pO2 (mm Hg)	62.2 ± 7.0	72.6 ± 6.4		NS
Venous pO2 (mm Hg)	16.3 ± 0.7	27.2 ± 0.8	+67%	<0.001
Arterial % Oxy-Hb Sat.	95.4 ± 0.8	95.3 ± 0.6		NS
Venous % Oxy-Hb Sat.	70.2 ± 1.7	64.4 ± 2.0	-8%	<0.05
Arterial O_2 Content (Vol. %)	13.6 ± 0.2	13.5 ± 0.2		NS
Venous O_2 Content (Vol. %)	10.1 ± 0.3	9.2 ± 0.3	-9%	<0.1
% O_2 Extraction ($\frac{A-V}{A}$ x 100)	26.2 ± 1.7	32.2 ± 2.4	+23%	<0.025
Myocardial O_2 Consumption (ml/min/100 gm)	6.40 ± 0.48	7.72± 0.77	+21%	<0.05
Arterial Lactate Conc. (mM)	4.28 ± 0.23 #	3.96± 0.27 #		NS
Venous Lactate Conc. (mM)	4.26 ± 0.23 #	3.92± 0.21 #		NS
A-V (mM)	0.02 ± 0.21	0.04± 0.1		NS
% Lactate Extraction ($\frac{A-V}{A}$ x 100)	+0.5%	+1.01%		NS

Table 2B (cont.)

Isoproteronol Stress (n=9)	High Oxy-Hb Affinity	Low Oxy-Hb Affinity	% Change	P^t
Arterial pO2 (mm Hg)	69.8 ± 8.0#	73.6 ± 6.3#		NS
Venous pO2 (mm Hg)	16.2 ± 1.2#	25.6 ± 0.8#	+58%	<0.001
Arterial % Oxy-Hb Sat.	95.3 ± 0.8#	95.8 ± 0.5#		NS
Venous % Oxy-Hb Sat.	66.4 ± 2.1*	60.2 ± 2.0*	-9%	<0.01
Arterial O_2 Content (Vol. %)	13.0 ± 0.2***	13.0 ± 0.2***		NS
Venous O_2 Content (Vol. %)	8.9 ± 0.3***	8.1 ± 0.2***	-9%	<0.05
% O_2 Extraction ($\frac{A-V}{A}$ x 100)	30.7 ± 2.0**	37.8 ± 2.0**	+23%	<0.005
Myocardial O_2 Consumption (ml/min/100gm)	7.41 ± 0.80#	9.27 ± 0.96*	+25%	<0.005
Arterial Lactate Conc. (mM)	4.62 ± 0.18 } #	4.19 ± 0.21 } #	-9%	<0.1
Venous Lactate Conc. (mM)	4.96 ± 0.27 }	4.40 ± 0.21 }	-11%	<0.025
A-V (mM)	-0.34 ± 0.18	-0.22 ± 0.22		NS
% Lactate Extraction ($\frac{A-V}{A}$ x 100)	-7.4%	-5.3%		NS

1. t: P for Low vs. High Oxy-Hb Affinity, paired t test

2. For isoproterenol vs. no isoproterenol hearts, and for arterial vs. venous lactate conc., # is P=NS, * is P<0.025, ** is P<0.01, *** is P<0.001, paired t test.

TABLE 3A

EFFECT OF OXY-HEMOGLOBIN AFFINITY STATE ON CONTRACTILE FUNCTION AND OXYGEN CONSUMPTION

Normothermic Series ($T=37^{o}C$)

	Basal State			
	High Affinity	Low Affinity	High Affinity	Low Affinity
Run no.: (n)	1 (12)	2 (12)	3 (11)[a]	4 (11)[a]
Heart Rate (beats/min)	180	180	180	180
LV Systolic Pressure (mm Hg)	71.8 ± 2.3	77.4 ± 2.4	70.2 ± 2.3	72.5 ± 2.7
LV Diastolic Pressure (mm Hg)	8.7 ± 0.6	9.5 ± 0.9	8.6 ± 1.0	8.4 ± 0.9
LV Developed Pressure (mm Hg)	63.1 ± 2.2	67.8 ± 2.4	61.5 ± 2.6	64.2 ± 2.6
Coronary Perfusion Pressure (mm Hg)	80.5 ± 10.0	89.9 ± 8.9	81.5 ± 11.7	90.9 ± 9.8
LV (+) dP/dt mm Hg/sec	988 ± 90	1075 ± 79	983 ± 98	1009 ± 91
LV (-) dP/dt mm Hg/sec	821 ± 63	873 ± 58	762 ± 54	802 ± 47
Double Product*	11360 ± 400	12210 ± 430	11080 ± 460	11550 ± 470
MVO_2 (ml O_2/100 gm/min)*	6.12 ± 0.41	8.45 ± 0.87	6.57 ± 0.58	7.64 ± 0.68

TABLE 3A (Cont.)

Normothermic Series (T=37°C)	Isoproterenol			
	High Affinity	Low Affinity	High Affinity	Low Affinity
Run no.: (n)	5 (6)	6 (6)	7 (5)[a]	8 (6)
Heart Rate (beats/min)	263 ± 21	276 ± 25	256 ± 27	272 ± 25
LV Systolic Pressure (mm Hg)	86.2 ± 4.9	93.8 ± 6.9	89.6 ± 5.3	93.7 ± 5.8
LV Diastolic Pressure (mm Hg)	9.2 ± 2.5	8.0 ± 1.6	17.4 ± 3.7	15.8 ± 3.2
LV Developed Pressure (mm Hg)	77.0 ± 5.3	85.8 ± 6.9	72.2 ± 4.3	77.8 ± 4.1
Coronary Perfusion Pressure (mm Hg)	76.0 ± 12.4	83.7 ± 8.2	84.8 ± 14.5	100.0 ± 8.5
LV (+) dP/dt (mm Hg/sec)	2050 ± 129	2429 ± 166	1982 ± 173	2204 ± 148
LV (-) dP/dt (mm Hg/sec)	1690 ± 124	1914 ± 141	1680 ± 167	1755 ± 133
Double Product*	19990 ± 1680	23280 ± 2160	18680 ± 2630	21060 ± 1960
MVO_2 (ml O_2/100gm/min)*	8.09 ± 1.40	10.7 ± 1.64	7.70 ± 1.73	8.88 ± 1.38

TABLE 3B

Hypothermic Series (T=30°C)	Basal State			
	High Affinity	Low Affinity	High Affinity	Low Affinity
Run no.: (n)	1 (8)[a]	2 (8)[a]	3 (8)[a]	4 (8)[a]
Heart Rate (beats/min)	90	90	90	90
LV Systolic Pressure (mm Hg)	111.3 ± 6.0	119.3 ± 5.9	107.3 ± 7.1	113.9 ± 7.4
LV Diastolic Pressure (mm Hg)	10.0 ± 0.6	9.8 ± 0.8	8.3 ± 0.6	9.6 ± 1.3
LV Developed Pressure (mm Hg)	101.3 ± 6.5	108.5 ± 7.2	99.0 ± 7.4	104.3 ± 8.1
Coronary Perfusion Pressure (mm Hg)	72.6 ± 11.0	90.4 ± 8.8	72.1 ± 10.9	87.5 ± 10.7
LV (+) dP/dt (mm Hg/sec)	886 ± 59	1023 ± 95	890 ± 69	940 ± 71
LV (-) dP/dt (mm Hg/sec)	530 ± 57	535 ± 54	535 ± 63	509 ± 56
Double Product*	9320 + 570	10000 ± 590	9220 ± 590	9710 ± 670
MVO_2 (ml O_2/100gm/min)*	6.45 ± 0.60	7.03 ± 0.71	5.78 ± 0.53	7,27 ± 0.51

TABLE 3B (Cont.)

Hypothermic Series (T=30°C)	Isoproterenol			
	High Affinity	Low Affinity	High Affinity	Low Affinity
Run no.: (n)	5 (9)	6 (9)	7 (9)	8 (9)
Heart Rate (beats/min)	143.3 ± 5.5	151.1 ± 5.3	139.7 ± 7.4	146.7 ± 4.2
LV Systolic Pressure (mm Hg)	102.7 ± 7.8	119.1 ± 7.9	96.1 ± 8.3	110.7 ± 8.3
LV Diastolic Pressure (mm Hg)	8.3 ± 1.4	8.4 ± 1.1	11.0 ± 2.2	11.8 ± 2.9
LV Developed Pressure (mm Hg)	94.2 ± 8.5	110.8 ± 8.6	85.1 ± 9.0	98.9 ± 8.7
Coronary Perfusion Pressure (mm Hg)	58.1 ± 10.2	68.1 ± 10.5	67.7 ± 12.7	74.2 ± 13.0
LV (+) dP/dt (mm Hg/sec)	1228 ± 115	1586 ± 119	1153 + 119	1408 ± 146
LV (-) dP/dt (mm Hg/sec)	766 ± 94	852 ± 98	699 ± 98	793 ± 93
Double Product*	13340 ± 1120	16690 ± 1330	11850 ± 1270	14510 ± 1350
MVO_2 (ml O_2/100gm/min)*	7.36 ± 0.82	9.57 ± 1.00	7.47 ± 0.81	8.96 ± 0.94

Data are shown (Mean ± SEM) for each run on high or low oxy-hemoglobin affinity red blood cells during the basal and isoproterenol states. The hearts had two runs on each type of red blood cell during both the basal and isoproterenol states. The sequence of experiments was systematically varied so that the first run alternated between high and low oxy-hemoglobin affinity red blood cells.
*Double Product + Heart Rate X Developed Pressure; MVO_2 = Myocardial Oxygen Consumption.
a: The pressure tracing from one heart could not be accurately analyzed because of frequent premature beats.

Statistical analysis of differences between the high and low affinity perfusion runs is reported in Figures 2,3, 5 and 6. Significant statistical differences between the basal state and the isoproterenol stress state are discussed in the text.

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